

Application No.:
Notice Dated:
Reply to Notice mailed:

09/712,768
July 21, 2004
July 30, 2004



AMENDMENTS TO THE CLAIMS

LISTING OF CLAIMS:

Claim 1. (Withdrawn) A cytochrome c oxidase complex having cytochrome c oxidase activity, which complex is obtainable by the isolation from a *Gluconobacter oxydans* DSM 4025 microorganism.

Claim 2. (Withdrawn) A cytochrome c oxidase complex according to claim 1, wherein the microorganism is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 3. (Withdrawn) A cytochrome c oxidase complex according to claim 1, wherein the complex has the following properties:

(a) comprising at least two core subunits of I (COI) and II (COII), wherein the apparent molecular mass of COI and COII are about 43 \pm 10 kDa and 36 \pm 10 kDa, respectively by SDS-PAGE; and

(b) providing an absorption spectrum showing an aa3-type cytochrome c oxidase peak at 605 \pm 1 nm in reduced minus oxidized difference spectrum.

Claim 4. (Withdrawn) A cytochrome c oxidase complex according to claim 1, wherein the isolated complex is substantially homologous to a native cytochrome c complex

Application No.:	09/712,768
Notice Dated:	July 21, 2004
Reply to Notice mailed:	July 30, 2004

from *Gluconobacter oxydans* DSM 4025 or a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 5. (Withdrawn) A cytochrome *c* oxidase complex according to any one of claims 1-4, which is a recombinant enzyme.

Claim 6. (Withdrawn) A cytochrome *c* oxidase complex according to claim 5 comprising a core subunit containing the amino acid sequence of SEQ ID NO: 2.

Claim 7. (Withdrawn) A cytochrome *c* oxidase complex according to claim 6 comprising an amino acid sequence having 85% or greater sequence identity with SEQ ID NO: 2, and having cytochrome *c* oxidase activity.

Claim 8. (Withdrawn) A cytochrome *c* oxidase complex according to claim 5 comprising at least one amino acid sequence selected from the group of SEQ ID NO: 4, 6 or 8.

Claim 9. (Withdrawn) A cytochrome *c* oxidase complex according to claim 8, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 4, 6 or 8, and is capable of providing the complex with cytochrome *c* oxidase activity.

Application No.:	09/712,768
Notice Dated:	July 21, 2004
Reply to Notice mailed:	July 30, 2004

Claim 10. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

Claim 11. (Withdrawn) A recombinant polypeptide according to claim 10, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 2, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 12. (Withdrawn) A recombinant polypeptide according to claim 10, which is encoded by the polynucleotide sequence of SEQ ID NO: 1.

Claim 13. (Withdrawn) A recombinant polypeptide according to claim 12, wherein the polynucleotide sequence encodes SEQ ID NO: 2 or an amino acid sequence having at least 85% identity with SEQ ID NO: 2 and being capable of providing the complex with cytochrome c oxidase activity.

Claim 14. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 4.

Claim 15. (Withdrawn) A recombinant polypeptide according to claim 14, wherein the polypeptide has an amino acid sequence that is at least 85% identical to SEQ ID NO: 4, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Application No.:	09/712,768
Notice Dated:	July 21, 2004
Reply to Notice mailed:	July 30, 2004

Claim 16. (Withdrawn) A recombinant polypeptide according to claim 14, which is encoded by a polynucleotide sequence of SEQ ID NO: 3.

Claim 17. (Withdrawn) A recombinant polypeptide according to claim 16, wherein the polynucleotide encodes SEQ ID NO: 4 or an amino acid sequence having at least 85% identity with SEQ ID NO: 4 and being capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

Claim 18. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence of SEQ ID NOs: 6 or 8.

Claim 19. (Withdrawn) A recombinant polypeptide according to claim 18, wherein the amino acid sequence is at least 85% identical to either SEQ ID NOs: 6 or 8, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome *c* oxidase activity.

Claim 20. (Withdrawn) A recombinant polypeptide according to claim 18, which is encoded by a polynucleotide selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 7.

Claim 21. (Withdrawn) A recombinant polypeptide according to claim 20 capable of providing the complex in any one of claims 1 - 9 with cytochrome *c* oxidase activity,

Application No.: 09/712,768
Notice Dated: July 21, 2004
Reply to Notice mailed: July 30, 2004

which is encoded by a polynucleotide selected from the group consisting of a polynucleotide encoding SEQ ID NO: 6, a polynucleotides encoding SEQ ID NO: 8, a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 6, and a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 8.

Claim 22. (Previously amended) A recombinant DNA comprising the polynucleotide sequence of SEQ ID NO: 1.

Claim 23. (Previously amended) A recombinant DNA comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2.

Claims 24-26. (Cancelled).

Claim 27. (Previously cancelled).

Claims 28-29. (Cancelled).

Claim 30. (Previously cancelled).

Claim 31. (Currently amended) An expression vector comprising a recombinant DNA according to any one of claims 22, 23 and 57 ~~22-26, 28 and 29~~, wherein the expression vector is suitable for expression in an organism.

Application No.: 09/712,768
Notice Dated: July 21, 2004
Reply to Notice mailed: July 30, 2004

Claim 32. (Previously cancelled).

Claim 33. (Currently amended) An expression vector according to claim 31, wherein the expression vector is a bacterial expression vector ~~organism is a microorganism~~.

Claims 34-37. (Cancelled).

Claim 38. (Currently amended) A host cell ~~recombinant microorganism~~ comprising the expression vector of claim 31.

Claim 39. (Cancelled).

Claim 40. (Currently amended) A host cell ~~recombinant microorganism~~ comprising at least one recombinant DNA according to any one of claims 22, 23 and 57 ~~22-26, 28- and 29~~.

Claim 41. (Previously cancelled).

Claim 42. (Currently amended) A host cell ~~recombinant microorganism~~ according to claim 40, wherein the host cell ~~microorganism~~ is a bacterium ~~bacteria~~.

Application No.: 09/712,768
Notice Dated: July 21, 2004
Reply to Notice mailed: July 30, 2004

Claim 43. (Currently amended) A host cell ~~recombinant microorganism~~ according to claim 40, which ~~42, wherein the microorganism~~ is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 44. (Currently amended) A host cell ~~recombinant microorganism~~ according to claim 40, wherein the host cell is ~~microorganism is obtained from~~ *Gluconobacter oxydans* DSM 4025.

Claim 45. (Cancelled).

Claim 46. (Currently amended) A process for producing a cytochrome c oxidase complex comprising:

- (a) cultivating in a culture medium a host cell ~~recombinant microorganism~~ according to claim 40; and
- (b) recovering a cytochrome c oxidase complex from the culture.

Claim 47. (Currently amended) A process according to claim 46, wherein the host cell ~~recombinant microorganism~~ is a bacterium ~~bacteria~~.

Claim 48. (Currently amended) A process according to claim 46, wherein the host cell ~~microorganism~~ is selected from the group consisting of *Escherichia coli*, *Pseudomonas*

Application No.: 09/712,768
Notice Dated: July 21, 2004
Reply to Notice mailed: July 30, 2004

putida, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 49. (Currently amended) A process according to claim 46, wherein the host cell ~~is microorganism is obtained from~~ *Gluconobacter oxydans* DSM 4025.

Claim 50. (Cancelled).

Claim 51. (Withdrawn) A process for producing 2-keto-L-gluconic acid (2-KGA) from L-sorbose or D-sorbitol comprising:

(a) cultivating in a culture medium a recombinant microorganism comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment of SEQ ID NO: 3, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, the polynucleotide fragment of SEQ ID NO: 5, a polynucleotide fragment that encodes the amino acid of SEQ ID NO: 6, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, a polynucleotide fragment of SEQ ID NO: 7, and a polynucleotide fragment that encodes the amino acid sequence of

Application No.: 09/712,768
Notice Dated: July 21, 2004
Reply to Notice mailed: July 30, 2004

SEQ ID NO: 8, and a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8 and capable of expressing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity; and

(b) recovering 2-KGA from the culture medium.

Claim 52. (Withdrawn) A process according to claim 51, wherein the recombinant microorganism is a bacteria.

Claim 53. (Withdrawn) A process according to claim 52, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 54. (Withdrawn) A process according to claim 53, wherein the microorganism is *Gluconobacter oxydans* DSM 4025.

Claim 55. (Withdrawn) A process according to claim 54, wherein the microorganism is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 56. (Withdrawn) A cytochrome c oxidase complex comprising a core subunit containing a polypeptide sequence selected from the group consisting of SEQ ID NO:2,

4, 6 and 8, fragments of SEQ ID NO:2 capable of providing the said complex with cytochrome c oxidase activity, and a polynucleotide sequence that encodes a polypeptide that is capable of providing the complex with cytochrome c oxidase activity, and which polynucleotide hybridizes under high stringency hybridization and wash conditions to a polynucleotide sequence encoding SEQ ID NO:2, 4, 6 or 8.

Claim 57. (Currently amended) A recombinant DNA comprising a polynucleotide sequence that hybridizes to a the complementary strand of SEQ ID NO: 1 under high stringency conditions comprising overnight incubation in 6X SSC, 0.5% SDS, 100 ug/ml denatured salmon sperm DNA, 50% formamide, at 42°C; followed by a first wash in 2X SSC, 0.5% SDS at room temperature for 15 minutes; followed by a second wash in 0.1X SSC, 0.5% SDS at room temperature for 15 minutes, wherein the recombinant DNA encodes a polypeptide that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* DSM 4025 cytochrome c oxidase core subunit II.

Claims 58-59. (Previously cancelled).

Claim 60. (Cancelled).

Claim 61. (Currently amended) A recombinant DNA according to claim 57, wherein the complex comprises at least a core subunit I (COI) and a core subunit II (COII), wherein the apparent molecular masses of COI and COII are about 43 ± 10 kDa and 36 ± 10 kDa, respectively as determined by SDS-PAGE and the complex has displays an

Application No.: 09/712,768
Notice Dated: July 21, 2004
Reply to Notice mailed: July 30, 2004

absorption spectrum ~~with a showing an aa3-type cytochrome c oxidase~~ peak at 605 ± 1 nm in a reduced minus oxidized difference spectrum.

Claim 62. (Cancelled).

Claim 63. (Currently amended) A recombinant DNA according to claim 57 24, wherein core subunit II comprises SEQ ID NO: 4.

Claims 64-72. (Cancelled).

Claim 73. (Currently amended) An expression vector comprising a ~~at least one~~ recombinant DNA according to claim 61 ~~any one of claims 61, 65 and 69~~.

Claims 74-75. (Cancelled).

Claim 76. (Currently amended) A host cell ~~recombinant microorganism~~ comprising a ~~at least one~~ recombinant DNA according to claim 61 ~~any one of claims 61, 65 and 69~~.

Claims 77-80. (Cancelled).

Claim 81. (New) A host cell comprising the expression vector of claim 73.